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Method for rearing the orb-web spider *Cyclosa argenteoalba*

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Abstract — Rearing of animals from one generation to the next is important in many biological studies. In this study, we aimed to develop efficient methods for rearing multiple generations of orb-web spiders (*Cyclosa argenteoalba*), testing three methods. First, we examined isolated rearing with aphid supplied by hand. Second, we tested mass rearing. We also collected various insect species from the field using light trap and supplied them by hand. Both methods failed, perhaps because of the inappropriate aphid (*Uroleucon formosanum*) and insufficient quantity of prey supplied in the first and second tests, respectively. For the third method, we mass reared with abundant prey directly supplied from light traps into the terrariums located outside. This method successfully raised hatchlings to adults, and the eggs produced from these adults also matured. The development time of wintering and non-wintering cohorts was 150.2−195.0 days and 24.0−76.3 days, respectively.

Key words — hatchling, isolated rearing, mass rearing, light-trap

Introduction

Rearing animals from one generation to the next is an essential technique in biological studies. Genetic studies require the knowledge of parent-offspring relationships among subject individuals, and the maintenance of genetic lineages. In physiology or ethology, recent studies stressed the importance of maternal effect, implying that the control of the environment of mother animals is required to answer various questions in offspring. Species for which rearing methods have successfully been established contribute to the development of various branches of biology as model species (e.g., mouse: Shrwin 2004; guppy: Burns 2008; fruitfly: Barth et al. 1997; jumping spider: Carducci & Jakob 2000).

Laboratory method for rearing multiple generations has been established in many spider taxa, including salticid, lycosid and linyphiid spiders (Li & Jackson 2003; Dinter 2004; Moreira & Del-Claro 2011). However, to our knowledge, detailed reports on those of orb-web spiders are scarce. Many studies had successfully maintained adult orb-web spiders for prolonged time periods. These studies often used flat cuboid boxes standing perpendicularly, in which spiders were individually placed and where they built their two-dimensional vertical orb-webs (Zschokke & Herberstein 2005). Conversely, the rearing of spiderlings appears relatively more challenging. The key difficulty may lie in providing an appropriate space for web construction and food supply. Zschokke and Herberstein (2005) proposed to place the freshly hatched cocoon into a container with supports for the webs, such as wood wool, and to add cultures of *Drosophila* flies or Collembola springtails as food. Walter et al. (2005) collectively maintained 20 *Argiope bruinnichi* spiderlings within large terrariums. They regularly released 45−50 fruitflies per day into the terrarium as prey; however, mortality of the spiderlings was high. Alternatively, Mayntz (2003) managed to raise *Zygiella x-notata* hatchlings to adulthood by individually supplying CO_2 anesthetized fruitflies on threads spun inside the tube. The study by Mayntz (2003) indicates that rearing spiders for multiple generations would also be possible in orb-web spiders. However, the method used in Mayntz (2003) was somewhat labor-intensive. We consider that the development of an easier method that can be applied to a wide array of orb-web spider species would be beneficial.

The purpose of this study is to develop efficient methods for rearing multiple generations of orb-web spiders. The test species used was *Cyclosa argenteoalba*. The spiders build their vertical orb-webs (approximately 20 cm diameter for adult webs) in bamboo and wooden forest or along architectures in human-residential areas. They are multivoltine and occur from April to December in Japan (Miyashita 1999). Adult females of this species inevitably require webs to capture prey.

In the current study, we captured matured females of *C. argenteoalba* from the field and obtained egg sacs. We then raised hatchlings (total body length was $\leq$1 mm) to adults under artificial conditions. We tested three rearing methods, and compared the rearing success and efficiency among them.

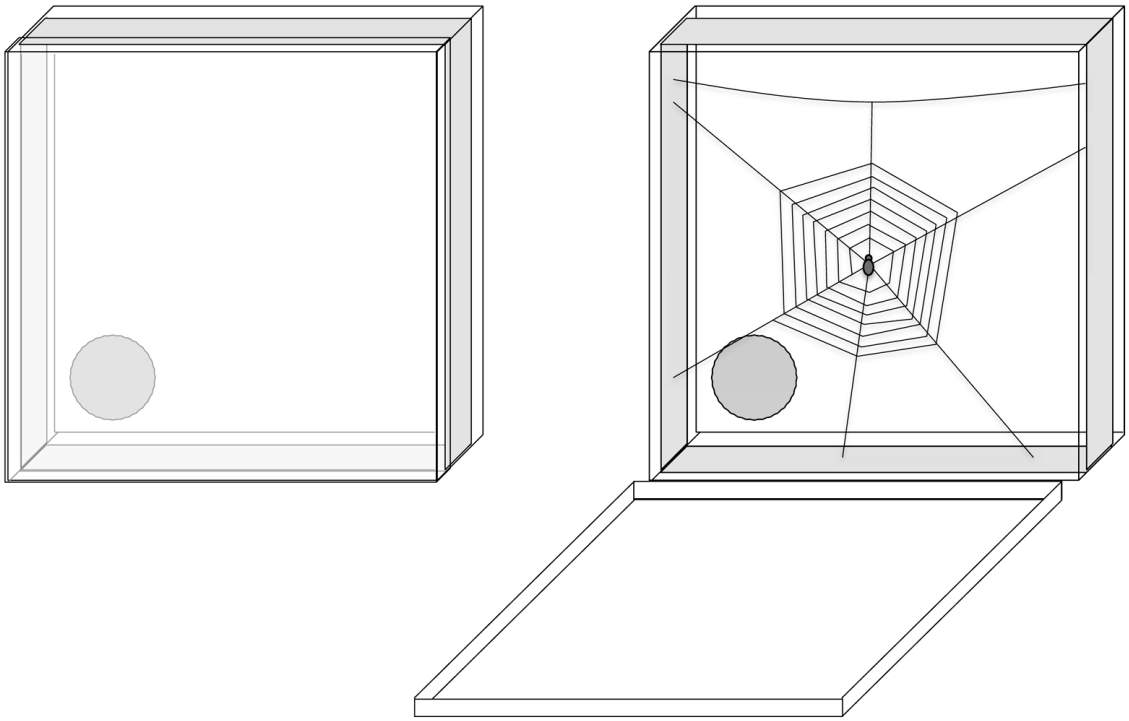

Fig. 1. Normal size raising cage of transparent polystyrene box for isolated rearing in method 1. Paper tape to facilitate grip for spiders and wet filter paper for humidity control are colored in gray. As shown in the right side, the front cover can be detached and prey items easily placed on the web.

1. Isolated rearing in the laboratory, with prey items supplied on the web by hand

Methods

We firstly referred the method used in Mayntz et al. (2003, 2009) who successfully raised *Z. x-notata* from hatchlings by maintaining individual spiders within a small cage, and live insects were supplied on their webs by hand. Although the method was proven to be successful in *Z. x-notata*, isolated rearing is expected to incur high logistical costs and may be inapplicable to *C. argenteoalba*. We aimed to examine the feasibility of the method by Mayntz et al (2003, 2009) with some modification of prey species. We tested several potential prey species, including a conspecific spiderling, used to examine the effect of cannibalism.

We collected matured females of *C. argenteoalba* on September 29, 2011 in bamboo and wooden forest in Nagaokakyo City, Kyoto. Spiders were promptly brought to the laboratory located in Nagasaki, Japan. The laboratory was maintained under a natural day-night cycle, whereas room temperature and humidity were not controlled. Spiders were kept in grass tubes (diameter: 21 mm, height: 40 mm) or small polystyrene boxes (14×14×3.5 cm) in which they laid their eggs for several days after the collection. Eggs were also maintained in the laboratory before hatchlings emerged from egg sacs (from October 16 to November 7).

In the laboratory, we used flat polystyrene boxes (14×14×3.5 cm) as normal size rearing cages, in which the inside was lined with paper tapes to allow grip. We also placed wet filter paper (diameter 5.5 cm) inside the cages for humidity control (Fig. 1). In addition, we used cages of different sizes (22×16×4.3 cm, n=4 and 11.3×10.5×2.8 cm, n=4), because preliminary study showed that cage size did not affect the rearing efficiency.

Mayntz (2003) stressed that the supplied prey items should be selected carefully as not to be too large for hatchlings to feed on. To locate prey of suitable size, we performed a preliminary investigation for several possible prey species from June to September in 2011. Initially, we used cultivated fruitflies (*D. melanogaster*) as prey. Flies were rendered inactive by cooling in a refrigerator and were gently placed on the web with fine forceps. However, the spiderlings removed the flies from the webs. Although we tried this method more than ten times, we never observed spiderlings handling the prey items for feeding. Subsequently, we collected soil-based insects such as springtails, nematode worms, and earthworms using a Tullgren funnel. However, we could not successfully utilize them as prey species due to their unsuitable size categories, with some species being too small to place on the web, and others too large to be captured by the spiderlings. We attempted to set a Tullgren funnel above the cage, allowing soil animals to fall directly onto the web threads. This method was not successful because large animals broke through the web whereas small animals were not

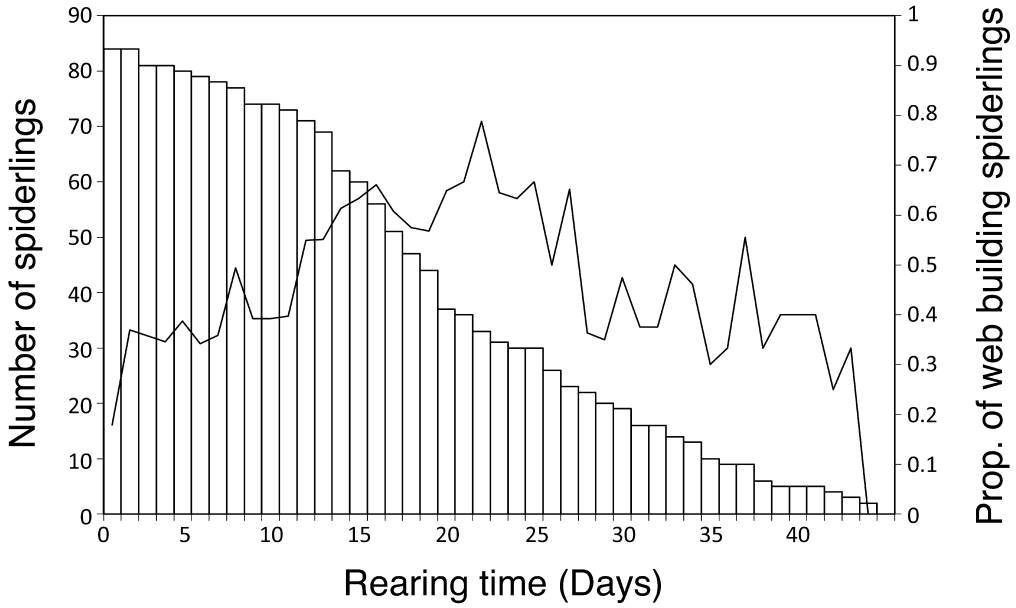

Fig. 2. The number of living spiderlings (bar graph) and the proportion of web building spiderlings (line graph) through rearing time in method 1. Rearing time is defined as the number of days subsequent to placing hatchlings into the cages, regardless of their hatching date.

intercepted. Finally, we used aphids (*Uroleucon formosanum*) as prey because wingless larva of appropriate size were abundantly available from the vegetation near the laboratory, and their slow movements allowed easy handling. In examinations, the spiderlings approached the aphids placed on the web and appeared to bite them. Therefore, we specifically aimed to study the effectiveness of aphids as food.

In addition to supplying aphids, we examined the influence of adding another spiderling into the cage as a prey species (hereafter termed 'coupled rearing'), because cannibalism is widespread in spiders and may be an important source of energy during periods of prey shortage (Toft & Wise 1999). The occurrence of cannibalism has not been reported in *C. argenteoalba*, but if it occurs, the survival rate of reared individuals will increase.

At the start of rearing experiment, we divided 84 hatchlings into 41 coupled rearing cages (i.e., two spiders per cage) and two isolated rearing cages (one spider per cage). We daily counted the number of living spiders in each cage and every morning checked whether the individual spiderlings had constructed a web. When a web was present, we placed one or two aphids on the web. Because cannibalism had not yet occurred after several days (3 to 18 days) and isolated rearing could yield a higher survival rate, 20 cages of coupled rearing were separated into 40 cages of isolated rearing (normal size cages). To examine the effect of additional individuals in the cage on spiderling survival rate, we conducted survival analysis by STATA 12 (Stata Corp., Texas, USA), in which the numbers of spiderlings in the cage at the start of observation and following reduction caused by death or separation of cages were included as time-dependent covariates.

Results and discussion

The proportion of daily web building spiderlings was maintained at greater than 30% except during 3 of 44 days, and exceeded greater than 50% for two weeks (Fig. 2). Spiderlings responded to the aphids attached on the web, and sometimes made contact for minutes or wrapped the prey item with silk, suggesting that spiderlings firstly accepted aphids as prey items, perhaps due to appropriate size. However, we did not obtain any evidence of feeding, and spiderlings occasionally discarded prey items from the web. Eventually, no spiders survived beyond 45 days (Fig. 2). Raising spiderlings to adulthood did not succeed possibly because the supplied aphids were inappropriate prey in quality for *C. argenteoalba*, as the aphid *Aphis nerii* was poor quality prey for *Schizocosa* lycosid spiders (Toft & Wise 1999).

Raising spiderlings to adulthood did not succeed by isolated rearing with prey supplied by hand. Nevertheless, we considered that this method could have been promising if we had been able to supply appropriate and available prey. Developing methods to collect or cultivate and supply small prey would be helpful. However, without such innovations, this method is not currently applicable to *C. argenteoalba.*

We did not observe any evidence of cannibalism when we kept paired spiderlings (total number of observation days was 451). We did not detect significant differences in the survival rate between isolated and coupled rearings, irrespective of cage sizes (Log-rank test for equality of survivor functions, $\chi^2 = 1.96$, $p = 0.16$). This may imply that *C. argenteoalba* spiderlings do not cannibalize with each other, even in close spatial proximity. Therefore, we considered that mass rearing in which many spiderlings were placed together into one terrarium, as in Zschokke and Herberstein

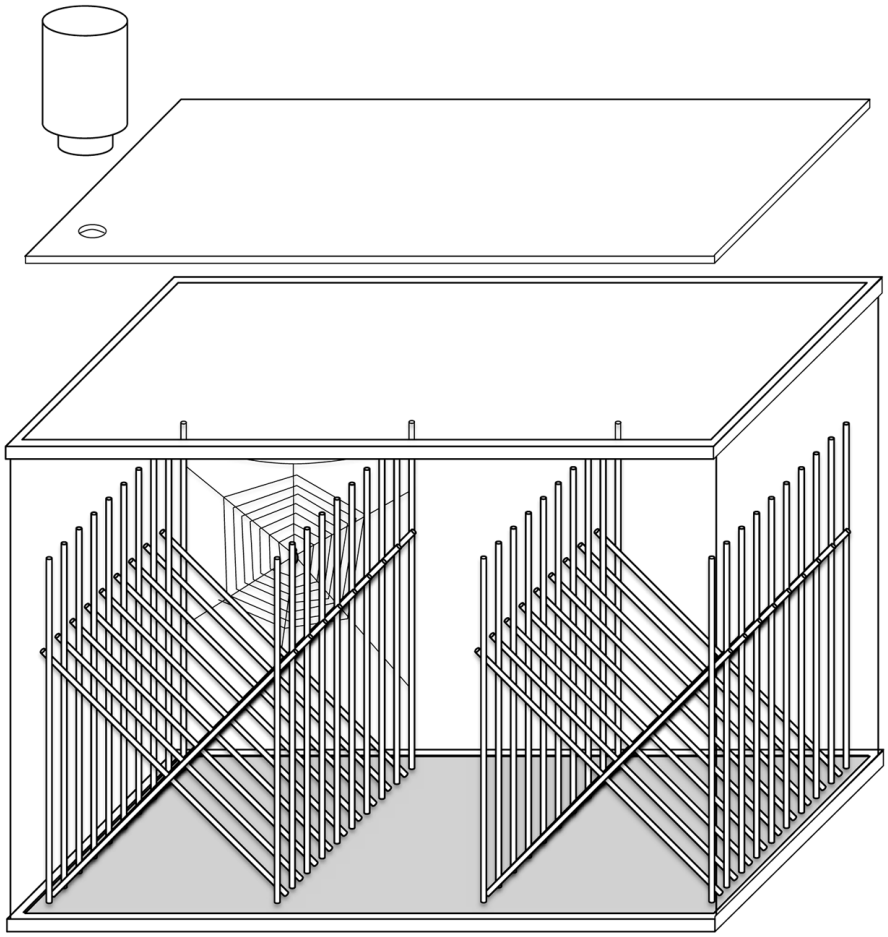

Fig. 3. Terrarium for mass rearing in the laboratory used in method 2. From top to bottom of the picture, the bottle containing prey items gathered by the light trap, the top cover of the terrarium with a closable opening to introduce prey items, and the terrarium with bamboo sticks stuck into the polystyrene as the attachment points and divider. Sticks in the central space in the terrarium were not drawn for simplicity.

(2005) and Walter et al. (2005), is also a feasible method.

2. Mass rearing in the laboratory, with prey items released into a terrarium by hand

Methods

Subsequent to the results of method 1, we tested mass rearing. A number of hatchling spiders from the same clutch were placed together into a middle-sized terrarium located in the laboratory. Prey insects were collected from a nearby field using light traps. We separated out small insects by covering a light trap with fine mesh, and regularly released prey items into the terrarium by hand. To facilitate the dense building of webs in a terrarium, we set a number of sticks as attachment points. The details are mentioned below.

A terrarium (28×40×25 cm, n=8) was equipped with bamboo sticks, some of which were stuck vertically into the polystyrene bottom sheet to act as attachment points of webs, whereas others were placed in an X-shape to minimize interference among spiderlings by sub-dividing the space inside the terrarium into 26 compartments (Fig. 3). Mature females were collected on May 24, 2012 in Kyoto and Osaka. The females laid eggs on May 25 and 26, and spiderlings hatched from June 6 to 8. We placed 57–116 hatchlings (mean=92.3) into each terrarium on June 6 and 11. The upper side of the terrarium was covered with a transparent sheet equipped with a closable opening (diameter 1.5 cm) for prey supply. Wet filter paper (diameter 5.5 cm) was placed into the terrarium to facilitate humidity control.

We collected prey insects by using UV emitting, AC powered, fluorescent light traps (SURE MC8200, Ishizaki Electric MFG. Co., Tokyo, Japan). The trap was equipped with a fan, and insects attracted to the light were trapped by air current produced by the fan. The insects were accumulated into a collecting net set below the fan. To separate out small insects, the light was covered with fine mesh (2.5 mm). In addition, a plastic bottle was attached under the collecting net to collect for weakened or dead insects that may fall in. Small dipteran and homopteran insects were mainly collected, such as midges and flies, small planthoppers, and winged aphids. We regularly checked the

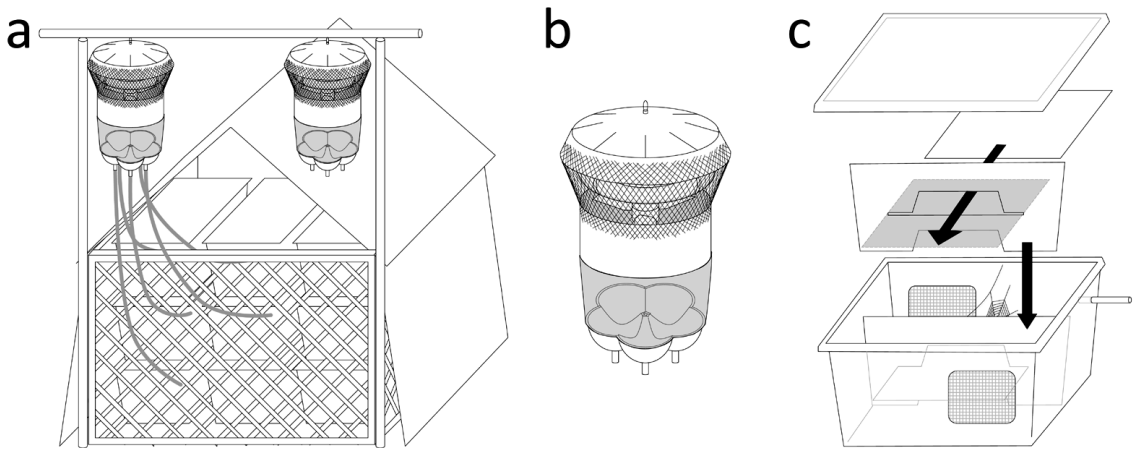

Fig. 4. Apparatuses for outdoor mass rearing in method 3, with prey items introduced from a light trap. a) The entire view showing the light trap connected by tubes to five raising cages and enclosed by a latticed wall and polypropylene covers. b) The light trap selectively collecting small prey items facilitated by the fine mesh net around the light, and fan under the light forcing prey items out from the five funnels. c) The raising cage containing spiderlings. From top to bottom, the top cover of the cage, the divider made of two polypropylene sheets, and the terrarium equipped with the divider, two rectangular openings for air current, and one opening connected to the light trap through a tube.

collecting net in the morning. Collected insects, if alive, were released into the terrarium. However, many insects were found dead. The dead insects were dropped directly onto the web.

Results and discussion

The number of spiderlings sharing a terrarium that concurrently (within a day) built webs was a maximum of five, indicating that the web-building rate was low using this method. Most spiderlings gathered along the underside of the top cover, and subsequently expired. Prey released into the terrarium by hand was seldom intercepted on the web, perhaps because their number was too low. Dead insects that were dropped onto the web, even if successfully intercepted on the web, were not consumed. As a result, we did not observe any evidence of spiders feeding on the prey supplied. No spiderling survived after 20 days from the start of the experiment.

Obviously, the reason for the low survival rate using this method lies both in the low web building rate and the low supply of prey. Too many bamboo sticks seemed to interfere with web building due to a scarcity of appropriate web space. Supplying prey by hand was also problematic as many prey insects died during handling, and spiderlings did not respond to dead prey. We conclude that we require the establishment of a method to supply a large number of live prey items and a larger web space.

The spiderling survival obtained in method 2 was worse than in method 1. This can likely be attributed to the difference in the season when experiments were conducted. While method 1 was conducted during winter, method 2 was conducted during summer when the metabolic rate of spiderlings was higher. Alternatively, in a terrarium, humidity may be less stable than the cages in method 1 because the same wet filter paper was used for humidity control, and desiccation may lead to a higher death rate.

3. Outdoor mass rearing, with prey items introduced from light traps

Methods

In the third and final method, to enable more efficient and abundant prey supply, we tested outdoor mass rearing. A number of hatchlings from the same clutch were placed together into a large-sized terrarium located outside. The terrarium was connected to a light-trap, from which collected insects were introduced into the terrarium without handling, significantly reducing the logistical costs.

We set terrariums constructed from translucent polypropylene and placed hatchlings inside (Fig. 4). Two sizes of terrariums ($26 \times 42 \times 31$ cm, $n=20$, and $26 \times 65 \times 31$ cm, $n=24$) were used. To retain an air current in the terrarium, two rectangular openings (15×15 cm) covered with 1 mm mesh were constructed, on the opposite sides of the terrarium. To minimize interference among spiderlings, we inserted several plastic sheets into the terrarium to sub-divide the inside space. Nevertheless, spiderlings were free to move to any location in the terrarium. We placed the terrariums under open sheds constructed of lattice fences and polypropylene boards to protect from rain and direct-sunlight (Fig. 4a).

We set the same light traps described previously over the shed, and connected the collecting net under light traps to the terrariums using transparent polypropylene tubes (diameter 12 mm, length $<$ 1.5 m) to introduce trapped insects into the terrariums by the air current produced by the fan. To selectively collect small insects, the light was covered with fine mesh (2.5 mm). Each light trap was connected with five terrariums. The entire experimental setup was located on the campus of Nagasaki Institute of Applied

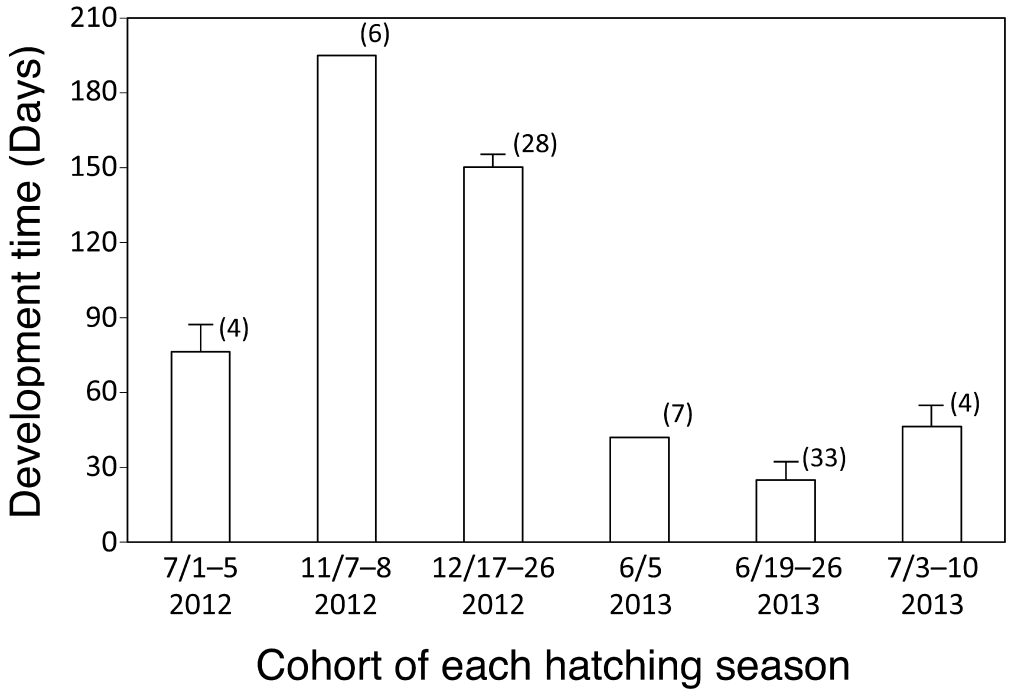

Fig. 5. The average time spent to maturation in the cohorts hatched during each season. The number in parentheses shows sample size, and the error bar shows standard deviation if it exists.

Science, with bushes and ponds providing abundant small insects. Lights were automatically activated from sunset to sunrise.

Mature gravid females were collected five times from June 2012 to June 2013 in Tokyo, Kyoto, and Osaka. Spiderlings were hatched from the produced eggs. We placed spiderlings from the same clutch into one to three terrariums, but never mixed spiderlings from different clutches into a common terrarium. The number of spiderlings per terrarium was counted only in 16 terrariums and ranged 6–66 (mean＝27.6). We checked the number and developmental status of spiders once or twice a week.

Results and discussion

The spiderlings constantly built their orb webs in the terrariums, but the number of webs was fewer than ten in most terrariums, implying that not all spiders are able to build webs in a terrarium of this size. Prey insects were constantly present in the terrariums, and we observed spiders making contact and feeding on prey. Non-prey insects, such as beetles, stink bugs, ants, and salticid and tetragnathid spiders also accidentally intruded into terrariums, perhaps through an aperture of the net covering the light trap.

In 15 of the 23 terrariums, we successfully raised spiderlings to adults. Subsequently, we outbred these spiders among clutches to obtain second generations and succeed in 15 of the 21 terrariums of them. Among 30 terrariums succeeded, the numbers of matured spiders ranged from 1 to 13 per terrarium (in 14 terrariums, only one adult was obtained), and the total number was 94 (28 males and 66 females). The proportion of hatchlings survived to adults, estimated from the terrariums where the number of hatchlings was counted, was 0.054 (24/442 spiders). The abdomen length of 17 gravid females was 4.88 mm (±0.65 SD) in average and ranged 3.62–5.69 mm. This was comparable to the previous description of this species, 2.38–4.63 mm (Tanikawa 1992). The nymphal development time varied among individuals, 21–195 days. We averaged it for each cohort considering hatching season (Fig. 5), found to range between 150.2–195.0 days for the wintering cohort and 24.0–76.3 days for the non-wintering cohort.

Method 3 was more successful than 1 and 2 as a rearing method. In actual fact, adults were obtained only through method 3. We consider that the reason for success in method 3 lies in the abundant prey supplied, which was realized by setting terrariums outdoors with connected light traps to introduce collected insects directly into the terrariums. This method seems to have a great advantage in decreasing logistical costs and in supplying various types of insects among which spiderlings can select preferable prey to feed on. The development time associated with method 3 varied, perhaps due to the seasonal effect on temperature and prey availability. The latter may also indicate the importance of prey supply in spider rearing.

General discussion

We tested three methods of rearing *C. argenteoalba* hatchlings to adults. In two methods among three, we set rearing cages or terrariums in a laboratory and supplied

field-collected prey by hand. Both of these methods failed, probably because of the insufficient prey supply and the difficulty in handling small prey. Mayntz et al (2003, 2009) had succeeded to rear spiders to adults using the isolated rearing method that is similar to method 1 in the current study. However, their subject spider, *Z. x-notata*, is able to feed on *Drosophila* flies, which are easily cultivated. Although the egg mass is ca. 0.15 mg in both *Z. x-notata* (Spilar 1992) and *C. argenteoalba* (Miyashita 1999), *C. argenteoalba* spiderlings could not feed on *Drosophila*, and we could not find an alternative prey that can be easily supplied by hand.

Conversely, outdoor mass rearing using in method 3 was more successful because prey items were supplied without handling and small insects, such as small midges, planthoppers, or flies, were trapped on webs. However, the efficiency was not always high, considering that no adults were raised in 14 terrariums among 44, and only a small proportion of hatchlings survived to adults if successful. This low efficiency may be attributed to the natural variation in prey supply, wet weather conditions, and the intrusion of non-prey large insects that destroyed webs. An improvement of rearing apparatus is required to minimize such ill-effects; however, it may incur additional costs.

Cultivating prey other than *Drosophila* may be another possible improvement to enhance the rearing efficiency. If this is realized, isolated rearing may be more fruitful, considering the higher web-building rate observed in this method. Nutrient imbalance from a homogeneous diet may result in problems; however, we expect that such problems will not be detrimental, considering the example of raising spiders by mono-species prey (Toft & Wise 1999). We consider that small midges are possible candidate for prey cultivation (Kawai & Imabayashi 2003).

In conclusion, we consider that our method described here is the first step to develop a more efficient method to rear multiple generations of orb-web spiders. Improvement of the method should be valuable for advancement of arachnological studies, particularly for orb-web spiders, in various aspects.

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